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Heterocyclic Nitrogen Compounds: Dibenz[a,j]acridine, Dibenz[a,h]acridine, 7H-Dibenzo[c,g]Carbazole

(Aza-Heterocyclics)

1937 The first investigation of the carcinogenicity of 7H-dibenzo[*c*,*g*]carbazole (DB[*c*,*g*]C) was conducted by Boyland and Brues. They found DB[*c*,*g*]C carcinogenic to mouse skin. [1]

1940 Badger and a group of associates, including Kennaway and Cook, found that of 10 mice given 5 milligrams of DB[*a*,*h*]Ac weekly, one mouse developed an epithelioma and papilloma of the forestomach; another mouse also showed stomach papillomas over a period of 627 days. In the same study, DB[*a*,*j*]Ac gave negative results.

In the introduction the authors state, "There is now little doubt that cigarette smoking is the major factor concerned with the ever-increasing incidence of bronchogenic carcinoma in men. Both cigarette-tobacco tars and particulates from air pollution contain various known carcinogenic polynuclear compounds." [2]

1940 Studies by Andervont and Shimkin demonstrated that all of 20 "A" mice given a single injection of 0.5 milligrams of DB[*a*,*h*]Ac in 0.1 milliliter tricaprylin developed multiple lung tumors but no local sarcomas by 14 weeks after injection, while all of 14 mice given a single injection of 1 milligram in 0.3 milliliter sesame oil developed multiple lung tumors but no local sarcomas 40 weeks after injection. Of 19 controls only 4 mice developed single lung tumors. [3]

1940 Badger *et al.*, in the study previously mentioned, demonstrated that of 10 mice given 5 milligrams of

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DB[*a,j*]Ac weekly (orally), no tumors were seen in 572 days. [4]

1951 Hartwell found DB[*a,j*]Ac to be carcinogenic to mouse skin and subcutaneous tissue of mice. [5]

1951 Hartwell determined that DB[*c,g*]C produced epithelioma in mice and sarcomas in rats and mice. [6]

1956 Lacassagne *et al.* found carcinogenic activity for DB[*a,j*]Ac when administered to the skin and subcutaneous tissues of mice. [7]

1957 Hartwell, along with Shubik further studied the previously determined carcinogenic effects of DB[*c,g*]C on rats and mice. [8]

1960 Van Duuren *et al.* distilled the basic portion of 250 grams of cigarette "tar" at 100° centigrade and 0.5 millimeters pressure. The residue (10 grams) was chromatographed on alumina and the benzene eluate repeatedly chromatographed on Whatman No. 1 paper and acetylated paper. Bands corresponding to that of the compounds dibenz[a,h]acridine (DB[*a,h*]Ac) and dibenz[*a,j*]acridine (DB[*a,j*]Ac) were rechromatographed until the band delivered ultraviolet absorption and fluorescence spectra that were comparable. Amounts of 0.27 micrograms of DB[*a,j*]Ac, 0.01 micrograms of DB[a,h]Ac and 0.07 micrograms of (DB[*c,g*]C) from the tar of 100 cigarettes were isolated. "It is clear that the heterocyclics here described make a significant contribution to the reported carcinogenicity of tobacco tars." [9]

1960 From the benzene eluate of the neutral portion of cigarette smoke condensate not found to be active on mouse skin by Wynder and Wright in their 1957 study, Van Duuren *et al.* isolated, by repeated paper chromatography, a compound identified as DB[*c,g*]C through comparison of radio frequency values,

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fluorescence, excitation and emission spectra. Van Duuren could not identify DB[*c,g*]C from cigarette smoke. [10]

1960 The Van Duuren *et al.* study demonstrated that *N*-heterocyclic hydrocarbons are formed during the pyrolysis of nicotine as well as pyridine; he demonstrated this for DB[*a,j*]Ac and DB[*a,h*]Ac through isolation from highly fluorescent pyrolizates obtained at 750° centigrade in nitrogen. The DB[*a,j*]Ac yield proved to be far higher than its isomer. [11]

1962 Methods for the separation and identification of DB[*c,g*]C in cigarette smoke were reported by Van Duuren. [12]

1963 Candeli *et al.*, with some modifications, applied the same method as Van Duuren, locating 2.0 micrograms of DB[*a,j*]Ac from 100 cigarettes. The presence of DB[*a,h*]Ac in cigarette smoke could not be confirmed. [13]

1963 Dibenz[*a,j*]acridine applied in 0.5 and 0.1% acetone solution thrice weekly to the backs of 20 Swiss female mice induced tumors after 12 to 14 months in 16 and 15 mice, respectively. In both groups, 60% of the mice developed carcinomas.

NOTE: According to Wynder and Hoffmann, "[T]his relatively high tumor response in mouse skin (compared to the finding of Lacassagne *et al.* in 1956) might be partially explained by the high purity of the compounds, due mainly to the absence of the 'Morgan's base' a dihydroacridine. Only by repeated column chromatography or alumina can a DB[*a,j*]Ac be isolated that is free of the dihydro product (absence of N-H band in infrared absorption spectrum.)" [14]

1963 In a study by Wynder and Hoffmann, mouse skin was initiated with a single application of 300 micrograms

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of DMBA and then painted with a 0.5% solution of DB[*a,j*]Ac, 3 times weekly. The same tumor response was observed as with the heterocyclic alone except that the tumors appeared 2 to 3 months earlier. "This result indicates that there is no significant tumor-promoting activity of DB[*a,j*]Ac despite its strong hyperplastic effect." [15]

1964 The report of the Advisory Committee to the Surgeon General identified DB[*a,h*]Ac, DB[*a,j*]Ac and DB[*c,g*]C as "carcinogenic polycyclic compounds isolated from cigarette smoke," found them "weakly carcinogenic" and listed them in amounts of 0.1, 2.7 and 0.7 micrograms per 1000 cigarettes, respectively. [16]

1965 Sawicki *et al.* identified DB[*a,h*]Ac and DB[*a,j*]Ac in polluted air in amounts of 0.08 and 0.04 micrograms/1000m^3 air. [17]

1969 Rothwell and Whitehead improved the method of isolation of DB[*c,g*]C from tobacco smoke condensate. [18]

1972 A study conducted by Sellakumar and Shubik demonstrated the high potent carcinogenic activity of DB[*c,g*]C in the respiratory system of 35 Syrian golden hamsters the test animals were given weekly instillations of 3 milligrams of DB[*c,g*]C suspended with an equal amount of haematite dust in saline, 30 developed 42 tumors in the respiratory tract, mainly squamous cell carcinomas and adenocarcinomas of the bronchial and tracheal epithelium. The first tumor appeared at 15 weeks. Of 45 hamsters given 30 similar weekly instillation of 0.5 milligrams, 40 developed 69 respiratory tumors, predominantly squamous cell carcinomas of the trachea, bronchi and larynx, with the first tumor appearing in the trachea at 30 weeks.

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In addition, the carcinogenic activity of DB[*c*,*g*]C was compared with that of BaP. It is interesting that 3 milligrams of DB[*c*,*g*]C, administered weekly for 15 weeks, produced 13% more animals dying with respiratory tract cancer than the same dose level of BaP. With lower dose levels (15 milligrams) of BaP only, 30% of the animals had respiratory tumors compared with 89% of the animals treated with DB[*c*,*g*]C; the tumors in the second group (DB[*c*,*g*]C) appeared earlier than those treated with BaP. [19]

1973 According to the IARC Working Group, skin painting with DB[*a*,*h*]Ac induces skin tumors in mice. Subcutaneous administration to mice induced local sarcomas and increased the incidence of lung tumors. In 1973, DB[*a*,*h*]Ac had not been tested by other routes or in other species. No human data was available "... however, coal tar and other materials which are known to be carcinogenic to man may contain DB[*a*,*h*]Ac." [20]

1973 The IARC Working Group concluded that DB[*a*,*j*]Ac induced skin tumors in mice following topical application and produced local sarcomas at the highest dose tested following subcutaneous application. It increased the incidence of lung tumors after subcutaneous administration. Negative results were obtained by the oral route in the mouse but the test was inadequate due to the small number of animals tested. It has not been tested in other species. No human data was available but some materials known to be carcinogenic to man may contain DB[*a*,*j*]Ac. [21]

1973 According to the IARC Working Group, DB[*c*,*g*]C is "carcinogenic in the mouse, rat, hamster and possibly in the dog. It has both a local and systemic carcinogenic effect. Following oral administration in the mouse, forestomach tumors and hepatomas occurred; intratracheal administration to hamsters produced tumors of the respiratory tract. In

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comparison with benzo[a]pyrene, DB[*c,g*]C appears to be a stronger respiratory tract carcinogen for the hamster."

Though DB[*c,g*]C had been detected in cigarette tar, no case reports or epidemiological data concerning exposure to humans were available. [22]

1976 In their article, "Respiratory Carcinogenesis," Wynder and Hoffmann listed D[*a,h*]Ac, DB[*a,j*]Ac and DB[*c,g*]C as "tumorigenic agents identified in the particulate phase of tobacco smoke, each possessing low biological activity and only traces detected in the smoke of 100 cigarette. DB[*a,j*]Ac was detected in amounts of 1.0 micrograms per 100 cigarettes. [23]

1976 Dipple, in "Polynuclear Aromatic Carcinogens" labeled DB[*a,h*]Ac, DB[*a,j*]Ac and DB[*c,g*]C as "carcinogenic heterocyclic compounds" with "slight" activity. [24]

1976 Wynder and Hoffmann categorized DB[*a,j*]Ac and DB[*a,h*]Ac as 'known animal carcinogens,' but further note that the aza-heterocyclics are 'minor carcinogens.' [25]

1977 Schmeltz and Hoffmann, in their review of the nitrogen-containing compounds in tobacco and tobacco smoke, describe DB[*a,h*]Ac, DB[*a,j*]Ac and DB[*c,g*]c as "animal tumor initiators." [26]

1979 The U.S. Surgeon General reports DB[*a,h*]Ac, DB[*a,j*]Ac and DB[*c,g*]C as carcinogenic azaarenes in cigarette smoke. [27]

1979 Rinkus et al. found DB[*a,h*]Ac and DB[*a,j*]Ac to be mutagenic substances. [28]

1982 The report of the U.S. Surgeon General identified DB[*a,h*]Ac, DB[*a,j*]Ac and DB[*c,g*]C as "tumor-initiating agents in the particulate phase of tobacco smoke" in

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amounts of 0.1, 3-10, and 0.7 nanograms per cigarette, respectively. While DB[*a*,*h*]Ac and DB[*c*,*g*]C possessed low relative carcinogenic activity, DB[*a*,*j*]Ac showed higher carcinogenic activity. [29]

1985 The IARC found sufficient evidence for the carcinogenicity to experimental animals for DB[*a*,*h*]Ac, DB[*a*,*j*]Ac and DB[*c*,*g*]C. [30]

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